IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	:	Group Art Unit 1642
MARK L. TYKOCINSKI et al.	:	Examiner Alana M. Harris, Ph.D.
Serial No. 09/957,056	:	Attorney Docket No. 285332-00002-2
Filed September 20, 2001	:	
CELLS AND VACCINES COMPRISING CELLS HAVING TRANSFERRED PROTEINS	:	

DECLARATION OF MARK I. GREENE

I, Mark I. Greene, being duly sworn hereby declare as follows:

1. I have an M.D. from the University of Manitoba, Canada, awarded in 1972.

2. From 1973 - 1976, I was a Fellow of the Royal College of Physicians, Canada, in the Department of Internal Medicine. I was awarded a Ph.D. from the University of Manitoba in Immunology in 1977. From 1972 - 1973 I was an Intern at the Health Sciences Centre, Winnipeg, Canada and from 1973 - 1976 I was a Resident at the Health Sciences Centre in Winnipeg, Canada. From 1973-1975 I received a Medical Research Council Fellowship, Canada; from 1976-1978 I was the recipient of a Medical Research Council Fellowship in Boston; and from 1976-1977 I was a Research Fellow in Pathology, Harvard Medical School, Boston, Massachusetts.

3. From 1977 to the present I have held various faculty appointments as listed in Exhibit A, most recently as Vice Chair of Pathology, Division of Immunology and Experimental Pathology, with the University of Pennsylvania (Philadelphia, PA).

4. I have received the awards and honors and am a member in the honorary societies listed in Exhibit B.

5. I have served on the various committees and held editorial positions listed in Exhibit C.

6. I am an author of the publications listed in Exhibit D.

7. I am a named inventor on the patents listed in Exhibit E and I have an inventor's understanding of the patent system.

8. I am not a named inventor on the captioned application. However, I have carefully reviewed the application, the outstanding Office Action issued in this case, and participated in the response to the written description rejection.

9. Claim 23, as amended, recites "An isolated cell having a lipidated protein incorporated into the cell membrane, said lipidated protein having bound thereto a fusion protein, said fusion protein consisting of a first domain and a second domain, said second domain encoding a protein having a costimulatory, inhibitory or adhesion function."

10. The three classes of proteins recited in Claim 23, namely costimulatory, inhibitory and adhesion proteins, are extremely well-known to those familiar with the art. Each class of protein has multiple members, which differ in molecular structure but share important functional properties.

11. "Costimulatory proteins" consist of membrane or soluble proteins that bind to cognate receptors, termed costimulator receptors, on immune cells, and via this binding event, provide activating signals to said immune cells. The majority of membrane-anchored costimulatory proteins belong to either the tumor necrosis factor superfamily (TNFSF) or the B7 family, but other membrane-anchored proteins also function as costimulatory proteins. In addition, a variety of soluble cytokines can provide costimulatory signals to immune cells. The structure of these proteins, the structure of their cognate receptors and the pathways by which each of these categories of costimulatory proteins activate immune cells is well known and was described in the literature prior to the filing date of the present application. See, for example, Greenfield, E.A., Nguyen, K.A., Kuchroo, V.K., "CD28/B7 costimulation: a review". *Crit. Rev. Immunol.* 18:389-418, 1998, which describes the CD28/B7 pathway in great detail, and includes a discussion of many of the various costimulator proteins described in the present application. The tumor necrosis factor superfamily has similarly been well-described.

12. It is my well considered opinion that one skilled in the art could easily determine if a particular protein of interest was a costimulatory protein as intended in the present application. Numerous assays exist, including the T-cell proliferation assay described in the application, to assess whether a particular protein functions as a costimulator.

13. In view of the above, it is my well-considered opinion that the inventors on this application had complete possession of the claimed invention. The term "costimulatory protein" adequately describes a well known category of proteins, the meaning of which is understood in the art and unambiguous, and for which assays exist to determine membership in this category.

14. Inhibitory proteins consist of membrane or soluble proteins that bind to cognate receptors, termed inhibitory receptors, on immune or other cells, and via this binding event, provide inhibitory signals to said cells. Inhibitory proteins can function to desensitize activation receptor signaling, induce immune cell anergy and proliferative inhibition, and/or induce apoptosis/cell death.

A well-studied subset of inhibitory proteins are those that bind to cognate inhibitory receptors with ITIM motifs. Other well-studied inhibitory proteins are those of the tumor necrosis factor superfamily (TNFSF), some of which induce immune cell apoptosis. As with the costimulatory proteins, the structure of these proteins, the structure of their cognate receptors, and the pathways by which each of these categories of inhibitory proteins inhibit or down-regulate immune cells is well known and was described in the literature prior to the filing date of the present application. See, by way of example, Long, E.O., "Regulation of immune responses through inhibitory receptors", *Annu Rev. Immunol.* 17:875-904, 1999; and Bolland, S., Ravetch, J.V., "Inhibitory pathways triggered by ITIM-containing receptors", *Adv. Immunol.* 72:149-177, 1999. Both of these articles describe in detail many inhibitory proteins, their structure and mechanism of action, all of which was known prior to the filing date of the present application.

15. It is my well considered opinion that one skilled in the art could easily determine if a particular protein of interest was an inhibitory protein as intended in the present application. Numerous assays exist, including the T-cell proliferation assay described in the application, and the annexin V-binding apoptosis assay, both of which are well known in the art, to assess whether a particular protein functions as an inhibitory protein.

16. In view of the above, it is my well-considered opinion that the inventors on this application had complete possession of the claimed invention. As was true for the term "costimulatory", the term "inhibitory protein" adequately describes a well known category of proteins, the meaning of which is understood in the art and unambiguous, and for which assays exist to determine membership in this category.

17. "Proteins having an adhesion function" consist of membrane proteins on one cell that bind to cognate receptors on a second cell, and via this binding event, promote adherence between said first and second cells. There are numerous examples of such proteins, including, but not limited to, integrins and selectins. This category of proteins was also well-characterized prior to the filing date of the present application. See, e.g.,

Shimizu, Y., Rose, D.M., Ginsberg, M.H., "Integrins in the immune system". *Adv. Immunol.* 72:325-380, 1999, which discusses one of the major classes of proteins having an adhesion function, integrins. Other classes of adhesion molecules were similarly well-described.

18. It is my well considered opinion that one skilled in the art could easily determine if a particular protein of interest was an adhesion protein as intended in the present application. Numerous assays exist, including cell:cell conjugate formation assays, to determine if a particular protein functions as an adhesion protein.

19. In view of the above, it is my well-considered opinion that the inventors on this application had complete possession of the claimed invention. As was true for the terms "costimulatory" and "inhibitory", the term "adhesion protein" adequately describes a well known category of proteins, the meaning of which is understood in the art and unambiguous, and for which assays exist to determine membership in this category.

20. It also my well-considered opinion that one skilled in the art would easily be able to carry out the full scope of Claim 23, without undue experimentation. The specification clearly describes how to make the fusion protein of interest and how to transfer this protein to a cell, as well as how to determine if the fusion protein has been successfully transferred, and provides numerous examples of how this was carried out in practice.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Aug 2/2004
Date

Mark I. Greene, M.D., Ph.D.

EXHIBIT A

FACULTY, HOSPITAL AND ADMINISTRATIVE APPOINTMENTS

Faculty Appointments:

1977-1978	Instructor in Pathology, Harvard Medical School
1978-1980	Assistant Professor in Pathology, Harvard Medical School
1980-1985	Associate Professor of Pathology, Harvard Medical School
1982-1985	Associate Professor of Immunology, Department of Cancer Biology, Harvard University
1984-1986	Professor of Medicine, Head: Rheumatology/Immunology, Tufts University
1986-present	Director, Division of Immunology, Department of Pathology Professor of Pathology, University of Pennsylvania
1987-present	Associate Director for Fundamental Research, Cancer Center, University of Pennsylvania
1989-present	John Eckman Professor of Medical Sciences, Department of Pathology and Laboratory Medicine, University of Pennsylvania
1993-present	Vice Chair of Pathology, Division of Immunology and Experimental Pathology, University of Pennsylvania

Hospital and Administrative Appointments:

1980-1986	Consultant in Medicine, Dana Farber Cancer Centre, Boston
1986 to present	Hospital of the University of Pennsylvania

Licensure:

1973	Canadian License Registration (Manitoba)
1976	Massachusetts License Registration (38692)
1976	Fellow of the Royal College (FRCP)
1985	Pennsylvania (M.D.-033875-E)

EXHIBIT B

AWARDS, HONORS AND MEMBERSHIP IN HONORARY SOCIATIES

1966	Memorial Scholarship
1966	Sir Sam Steele Memorial Scholarship
1966	Actuarial Award
1966	University of Manitoba Scholastic Award
1966	Mathematic Association Prize
1973-1978	Medical Research Council Fellowship Award
1982	American Cancer Society Faculty Award
1985	American Society for Clinical Investigation
1985-1987	Focused Giving Award, Johnson & Johnson
1986	Lotte Strauss Award
1988-1993	Markey Trust Award-Receptor Biology
1988-1990	Trustee: Leukemia Society of America
1989	Councilor-American Society for Clinical Investigation
1989	John Eckman Professor of Medical Sciences
1991-1992	John Guggenheim Fellow
1991-1992	American Cancer Society Annual Scientific Award
1993-1996	Human Frontiers Award
1994	Bride's Magazine: Cancer Research Award
1994	Dean's Award
1995	Interurban Clinical Club
1996	Capcure Award
1996	American Association of Physicians (AAP)
1998	Stanley N. Cohen Biomedical Research Award
1998	Abramson Family Cancer Research Award
1999	Newton Abraham Professor-Oxford University (2002-2003)
2002	Ashmolean Society
2003	Master of Arts (Hon) Oxford University

EXHIBIT C

MAJOR COMMITTEE ASSIGNMENTS (NATIONAL AND REGIONAL)

1975	RH Institute Awards Committee, Canada
1978	British Society of Immunology
1980	American Association Immunologists
1982	American Association of Pathologists
1982-1985	Massachusetts Medical Association
1986	Chairman; Department of Physiology Chair Search Committee
1989	Chairman; Structural and Molecular Biology at the University of Pennsylvania-Review Committee
1989	Howard Hughes Advisory Committee-University of Pennsylvania
1995-1999	Howard Hughes Review Committee
1996-1999	NIH-NIDCD, Board of Scientific Counselors
2001- present	Scientific Advisor- Roswell Park Memoral Cancer Insitute
2001- present	Scientific Advisor- Breast Cancer program-MD Anderson
2000-2005	Riken Institute, Board of Scientific Advisors

Editorial Positions:

Journal of Immunology, ad hoc reviewer
Journal of Experimental Medicine Cell , ad hoc reviewer
Nature, ad hoc reviewer
Science, ad hoc reviewer
Cellular Immunology-Editorial Board- through 1998
Immunologic Research-Editorial Board- present
EMBO , ad hoc reviewer
DNA & Cell Biology-Editor in Chief, 1990-Present
Journal of Mammary Gland Biology and Neoplasia Editorial board-present Experimental and Molecular Pathology- Senior Editor, 2000-Present
Pathobiology -Editor, 1990-1998

EXHIBIT D

PUBLICATIONS

1. Fujimoto, S., Greene, M.I. and Sehon, A.H.: Immunosuppressor T cells in tumor bearing hosts. Immunological Communications, 4(3):207-217, 1975.

2. Greenberg, A.H. and Greene, M.I.: Non-adaptive rejection of small tumor inocula as a model of immune surveillance. Nature, 264(5584):356-357, 1976.

3. Fujimoto, S., Greene, M.I. and Sehon, A.: Regulation of the immune response to tumor antigens. I. Immunosuppressor T cells in tumor-bearing host. Journal of Immunololology, 116(3):791-799, 1976.

4. Fujimoto, S., Greene, M.I. and Sehon, A.: Regulation of the immune response to tumor antigens. II. The nature of immunosuppressor cells in tumor-bearing hosts. Journal of Immunology, 116:800-806, 1976.

5. Greene, M.I., Fujimoto, S. and Sehon, A.: Regulation of the immune response to tumor antigens. III. Characterization of thymic suppressor factor(s) produced by the tumor-bearing host. Journal of Immunology, 119(2):757-764, 1977.

6. Greene, M.I., Pierres, A., Dorf, M.E. and Benacerraf, B.: The I-J subregion codes for determinants on suppressor factor(s) which limit the contact sensitivity response to picryl chloride. Journal of Experimental Medicine, 146:293-296, 1977.

7. Greene, M.I., Dorf, M.E., Pierres, M. and Benacerraf, B.: Reduction of syngeneic tumor growth by an anti-I-J alloantiserum. Proc. Natl. Acad. Sci. (USA), 74(11):5118-5121, 1977.

8. Greene, M.I., Sugimoto, M. and Benacerraf, B.: Mechanisms of regulation of cell-mediated immune responses. I. Effect of the route of immunization with TNP-coupled syngeneic cells on the induction and suppression of contact sensitivity to picryl chloride. Journal of Immunology, 120(5):1604-1611, 1978.

9. Perry, L., Benacerraf, B., McCluskey, R. and Greene, M.I.: Enhanced syngeneic tumor destruction by *in vivo* inhibition of suppressor T cells using anti-I-J alloantisera. American Journal of Pathology, 92:491-502, 1978.

10. Bach, B.A., Sherman, L., Benacerraf, B. and Greene, M.I.: Mechanisms of the regulation of cell-mediated immunity. II. Induction and suppression of delayed-type hypersensitivity to azobenzenearsonate-coupled syngeneic cells. Journal of Immunology, 121(4):1460-1468, 1978.

11. Perry, L., Benacerraf, B. and Greene, M.I.: Regulation of the immune response to tumor antigen. IV. Tumor antigen-specific suppressor factor(s) bear I-J determinants and induce suppressor T cells *in vivo*. Journal of Immunology, 121(6):2144-2147, 1978.

12. Greene, M.I. and Perry, L.: Regulation of the immune response to tumor antigen. VI. Differential specificities of suppressor T cells or their products and effector T cells. Journal of Immunology, 121(6):2363-2366, 1978.

13. Greene, M.I., Perry, L. and Benacerraf, B.: Regulation of the immune response to tumor antigen. V. Modulation of suppressor T-cell activity *in vivo*. American Journal of Pathology, 95:159-169, 1979.

14. Perry, L.L., Dorf, M.E., Benacerraf, B. and Greene, M.I.: Regulation of immune response to tumor antigen: Interference with syngeneic tumor immunity by anti-IA alloantisera. Proc. of Nat'l. Acad. of Sci. (USA), 76(2):920-924, 1979.

15. Chow, D.A., Greene, M.I. and Greenberg, A.H.: Macrophage dependent, NK-cell-independent "natural" surveillance of tumors in syngeneic mice. International Journal of Cancer, 23:788-797, 1979.

16. Bach, B.A., Greene, M.I., Benacerraf, B. and Nisonoff, A.: Mechanisms of regulation of cell-mediated immunity. IV. Azobenzenearsonate (ABA) specific suppressor factor(s) bear cross-reactive idiotypic determinants the expression of which is linked to the heavy-chain allotype linkage group of genes. Journal of Experimental Medicine, 149:1084-1098, 1979.

17. Weinberger, J.Z., Greene, M.I., Benacerraf, B. and Dorf, M.E.: Hapten-specific T-cell responses to 4-hydroxy-3-nitrophenyl acetyl. I. Genetic control of delayed-type hypersensitivity by V_H and I-A-region genes. Journal of Experimental Medicine, 149:1336-1348, 1979.

18. Finberg, R., Greene, M.I., Benacerraf, B. and Burakoff, S.J.: The cytolytic T lymphocyte response to trinitrophenyl modified syngeneic cells. I. Evidence for antigen specific helper T cells. Journal of Immunology, 123(3):1205-1209, 1979.

19. Finberg, R., Burakoff, S.J., Benacerraf, B. and Greene, M.I.: The cytolytic T lymphocyte response to trinitrophenyl modified syngeneic cells. II. Evidence for antigen-specific suppressor T cells. Journal of Immunology, 123(3):1210-1214, 1979.

20. Weinberger, J.Z., Germain, R.N. Ju, S.T., Greene, M.I. Benacerraf, B. and Dorf, M.E.: Hapten-specific T cell responses to 4-hydroxy-3-nitrophenyl acetyl. II. Demonstration of idiotypic determinants on suppressor T cells. Journal of Experimental Medicine, 150:761-776, 1979.

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22. Sy, M.S., Bach, B.A., Brown, A., Nisonoff, A., Benacerraf, B. and Greene, M.I.: Antigen- and receptor-driven regulatory mechanisms. II. Induction of suppressor T cells with idiotype-coupled syngeneic spleen cells. Journal of Experimental Medicine, 150:1229-1240, 1979.

23. Greene, M.I., Sy, M.S., Kripke, M. and Benacerraf, B.: Impairment of antigen-presenting cell function by ultraviolet radiation. Proc. Natl. Acad. Sci. (USA), 76(12):6591-6595, 1979.

24. Bach, B.A., Greene, M.I., Benacerraf, B. and Nisonoff, A.: Mechanisms of regulation of cell-mediated immunity. IV. Azobenzenearsonate-specific suppressor factor(s) bear cross-reactive idiotypic determinants the expression of which is linked to heavy-chain allotype linkage group of genes. Journal of Experimental Medicine, 149:1084-1098, 1979.

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26. Perry, L.L., Kripke, M., Benacerraf, B., Dorf, M.E. and Greene, M.I.: Regulation of the immune response to tumor antigen. VIII. The effects of host specific anti-I-J antibodies on the immune response to tumors of different origin. Cellular Immunology, 51:349-359, 1980.

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37. Letvin, N.L., Greene, M.I., Benacerraf, B. and Germain, R.N.: Immunologic effects of whole-body ultraviolet irradiation. Selective defect in splenic adherent cell function *in vitro*. Proc. Natl. Acad. Sci. (USA), 77(5):2881-2885, 1980.

38. Fox, I.J., Perry, L.L., Sy, M.S., Benacerraf, B. and Greene, M.I.: The influence of ultraviolet light irradiation on the immune system. Clinical Immunology and Immunopathology, 17:141-155, 1980.

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41. Letvin, N.L., Fox, I.J., Greene, M.I. and Germain, R.N.: Immunologic effects whole body ultraviolet (UV) irradiation. II. Defect in splenic adherent cell antigen presentation to primed T cells. Journal of Immunology, 125:1402-1404, 1980.

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45. Bhan, A.K., Perry, L.L., Cantor, H., Benacerraf, B. and Greene, M.I.: Role of T cell sets in the rejection of a methylcholanthrene-induced sarcoma (S1509a) in syngeneic mice. American Journal of Pathology, 102:20-27, 1981.

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54. Fox, I.J., Sy, M.S., Benacerraf, B. and Greene, M.I.: Impairment of antigen-presenting cell function by ultraviolet radiation. II. The effect of *in vitro* ultraviolet irradiation on antigen-presenting cells. Transplantation, 31(4):262-265, 1981.

55. Greene, M.I. and Sy, M.S.: Ligand-receptor relationships in immune regulation. Federation Proceedings, 40:1458-1461, 1981.

56. Sy, M.S., Brown, A., Bach, B., Benacerraf, B., Gottlieb, P., Nisonoff, A., and Greene, M.I.: Genetic and serologic analysis of the expression of crossreactive idiotypic determinants on anti-*p*-azobenzenarsonate antibodies and *p*-azobenzenarsonate-specific suppressor T cell factors. Proc. Natl. Acad. Sci. (USA), 78(2):1143-1147, 1981.

57. Whitaker, R.B., Nepom, J.T., Sy, M.S., Takaoki, M., Gramm, C.F., Fox, I.J., Germain, R.N., Nisonoff, A., Greene, M.I. and Benacerraf, B.: Suppressor factor from a T cell hybrid inhibits delayed hypersensitivity responses to azobenzenearsonate (ABA). Proc. Natl. Acad. Sci. (USA), 78:6441-6445, 1981.

58. Hirai, Y., Dohi, Y., Sy, M., Greene, M.I. and Nisonoff, A.: Suppressor T cells induced by idiotype-coupled cells function across an allotype barrier. Journal of Immunology, 126(5):2064-2066, 1981.

59. Schoen, R.T., Greene, M.I. and Trentham, D.E.: Antigen-specific suppression of type II collagen-induced arthritis by collagen-coupled spleen cells. Journal of Immunology, 128(2):717-719, 1982.

60. Nepom, J.T., Weiner, H.L., Dichter, M., Tardieu, M., Spriggs, D., Powers, L., Fields, B. and Greene, M.I.: Identification of a hemagglutinin-specific idiotype associated with reovirus recognition shared by lymphoid and neuronal cells. Journal of Experimental Medicine, 155:155-167, 1982.

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65. Ginsburg, C.H., McCluskey, R., Nepom, J.T., Takaoki, M., Falchuk, A.M., Benacerraf, B. and Greene, M.I.: Antigen- and receptor-driven regulatory mechanisms. X. The induction and suppression of hapten-specific granulomas. American Journal of Pathology, 106:421-431, 1982.

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EXHIBIT E

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